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INVITED REVIEW

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Abstract Endothelial cells are both the source and target of factors contributing to atherosclerosis. After the discovery of the endothelium-derived relaxing factor (EDRF) by Robert F. Furchgott in 1980 it soon became clear that endothelial cells also release vasoactive factors distinct from nitric oxide (NO) namely, endothelium-derived contracting factors (EDCF) as well as hyperpolarizing factors (EDHF). Vasoactive factors derived from endothelial cells include NO/EDRF, reactive oxygen species, endothelins and angiotensins which have either EDRF or EDCF functions, cyclooxygenase-derived EDCFs and EDRFs, and EDHFs. Endothelial factors are formed by enzymes such as NO synthase, cyclooxygenase, converting enyzmes, NADPH oxidases, and epoxigenases, among others, and participate in the regulation of vascular homeostasis under physiological conditions; however, their abnormal regulation due to endothelial cell dysfunction contributes to disease processes such as atherosclerosis, arterial hypertension, and renal disease. Because of recent changes in world demographics and the declining health status of the world's population, both aging and obesity as independent risk factors for atherosclerosis-related diseases such as coronary artery disease and stroke, will continue to increase in the years to come. Obesity and associated conditions such as arterial hypertension and diabetes are now also some of the primary health concerns among children and adolescents. The similarities of pathomechanisms activated in obesity and aging suggest that obesity—at least in the vasculature—can be considered to have effects consistent with accelerated, "premature" aging. Pathomechanisms as well as the clinical issues of obesity- and aging-associated vascular changes important for atherosclerosis development and prevention are discussed.

Keywords Aging · Vascular · Obesity · Nitric oxide · Endothelin · Endothelial cell · Vascular smooth muscle cell

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The discovery of endothelium-dependent control of vascular function

Endothelial cell research gained particular interest among physiologists and physicians only in the last 20 years of the twentieth century [46]. Endothelial cells form the inner lining of arterial and venous blood vessels and amount to approximately 1.5 kg, covering an area of approximately four tennis courts [78]. Under normal conditions, endothelial cells constantly produce a number of vasoactive and trophic substances that control inflammation, vascular smooth muscle cell growth, vasomotion, platelet function, and plasmatic coagulation [8, 129]. In the early 1970s, Ross and Glomset reported that endothelial cells exert a protective effect preventing smooth muscle cells to proliferate, which generated the "response-to-injury" theory of atherosclerosis [110], Since 1980, following the seminal observation of Robert F. Furchgott that endothelial cells release vasoactive factors that modulate vascular tone [47, 98], many advances have been made with regard to understanding how endothelial cell-derived factors both contribute to and interfere with the development of a number of cardiovascular pathologies [16, 129]. These factors, which are formed not only by endothelial cells but also by other cells such as vascular smooth muscle cells or

mesangial cells, are now known to contribute to the abnormal regulation of vascular tone and cell growth [7]. A selection of the most important factors identified so far that have been extensively studied in numerous physiological and pathophysiological conditions [134] have been summarized in Table 1. Research of the past three decades has provided evidence that inhibition of detrimental pathways such as blocking reactive oxygen species or receptors for endothelin or angiotensin, or increasing NO bioactivity can attenuate inflammation and subsequent disease progression and will have beneficial effects on disease outcome and survival [6, 16, 129].

Endothelium-derived vasodilators

The cyclooxygenase product, *prostacyclin* (prostaglandin I_2), was discovered in the early 1970s by Vane and associates [132]. Prostacyclin is formed by endothelial and other cells and has vasodilator and growth inhibitory activity [40, 90, 134]. The release of the *endothelium-derived relaxing factor (EDRF)* in response to acetylcholine was discovered by Furchgott in 1980, after both Vanhoutte [133] and Toda [126] in 1974 independently reported vascular relaxation in response to the muscarinic agonist. Furchgott provided proof that this vasorelaxation requires endothelial cells to release an unstable dilator factor [47]. The identity of *EDRF* as *nitric oxide (NO)* was independently demonstrated by Furchgott and by Ignarro in the mid-1980s [46, 63]. Endothelium-derived NO, formed by endothelial nitric oxide synthase (isoform 3, NOS3) by genomic and non-genomic mechanisms, as well as by a variety of post-translational modifications including phosphorylation [44], is not only a vasodilator but also inhibits cell growth and inflammation [49, 109]. Endothelium-dependent NO-independent dilatation is largely mediated by hyperpolarization, and a number of *endothelium-derived hyperpolarizing factors (EDHFs)* have been identified. They include epoxyeicosatrienoic acids (EETs), which are cytochrome P-450 metabolites, H_2O_2, endothelial gap junction communication, and potassium [reviewed in 27, 28]. Different EDHFs may also interfere with each other [74] (Table 1). *Endothelins* are endothelial cell-derived vasoactive peptides. Both ET-1 and ET-3 (see below) exert vasodilator activity through activation of endothelial cell ET_B receptors [7] and subsequent formation of NO [60]. *Angiotensins* are also formed by endothelial cells. Angiotensin II—through the endothelial AT_2 receptor [78]—and its break-down products, Ang 1-7, through its receptor MAS [112] and angiotensin IV (Ang 3-8)

Table 1 A selection of known endothelial cell-derived substances with either vasodilator or vasoconstrictor activity

Molecule	Source/enzyme	Target/receptor
Endothelium-derived vasodilators		
NO/EDRF	NOS3	VSMC, soluble guanylate cyclase
PGI_2/Prostacyclin	Cyclooxygenase-1 and 2	Prostacyclin receptor (IP)
EETs/EDHF	EDHF synthase/cytochrome P_{450} expoxygenase	VSMC, SK(Ca) and IK(Ca) channels
H_2O_2/EDHF	Catalase	VSMC, SK(Ca) and IK(Ca) channels
K^+/EDHF		VSMC, SK(Ca) and IK(Ca) channels
Gap junctions/EDHF		VSMC, TRPV4 and SK(Ca) channels
Endothelin-1	ECE-1, ECE-2 chymase, VSMC chymase	NOS3, EC endothelin ET_B receptor
Endothelin-3	ECE-1, ECE-3	NOS3, EC endothelin ET_B receptor
Angiotensin II	ACE	NOS3, EC angiotensin AT_2 receptor
Angiotensin 1-7	ACE2	NOS3, EC MAS receptor
Endothelium-derived vasoconstrictors		
Prostanoids/EDCF	Arachidonic acid; cyclooxygenase-1	VSMC; thromboxane receptor (TP)
Thromboxane A_2	Thromboxane synthase	VSMC; thromboxane receptor (TP)
O_2^-/superoxide	NADPH oxidase/NOX4	NO inactivation and $ONOO^-$ formation
O_2^-/superoxide	EDHF synthase/cytochrome P_{450} expoxygenase	NO inactivation and $ONOO^-$ formation
Endothelin-1	ECE-1, ECE-2	VSMC; endothelin ET_A receptor
Angiotensin II	ACE	VSMC; angiotensin AT_1 receptor

ACE angiotensin converting enzyme, *ACE2* angiotensin converting enzyme-2, *Cyt* cytochrome, *EC* endothelial cell, *ECE* endothelin converting enzyme, *EDHF* endothelium-derived hyperpolarizing factor, *EDRF* endothelium-derived relaxing factor, *EET* epoxyeicostrienoic acids, *IK(Ca)* intermediate conductance Ca(2+) activated K(+) channel, *VSMC* vascular smooth muscle cell, *NOS* NO synthase, *ONOO* peroxynitrite,.O_2^- superoxide anion, *TP* thromboxane receptor, *TRP* transient receptor potential, *SK(Ca)* small conductance Ca(2+) activated K(+) channel

through the AT_4 receptor [59, 102, 137], cause endothelium-dependent dilation, involving activation of ecNOS/cGMP.

Endothelium-derived vasoconstrictors

Arachidonic acid-derived *vasoconstrictor prostanoids* were the first endothelium-derived contracting factors *(EDCFs)* and identified by DeMey and Vanhoutte, who demonstrated contractile effects mediated by cyclooxygenase products shortly after the report of endothelium-dependent dilation [38]. *Superoxide anion*, a short-lived by-product of oxidative metabolism, was also found to have vasoconstrictor activity again by Vanhoutte's group [111] and also by Moncada and associates [56]. This effect is largely due to the EDRF/NO-inactivating properties of the superoxide anion [111]. The source of reactive oxygen species has been studied since the early 1990s and Griendling and coworkers have identified a vascular NADPH oxidase (NOX) as one of the major sources of vascular reactive oxygen species [55]; the NOX4 isoenzyme is mainly expressed in endothelial cells [24]. Interestingly, EDHF synthase/cytochrome P450 expoxygenase is also a source of superoxide anion [43]. In the mid-1980s several groups reported the release of a potent peptidergic vasoconstrictor substance from endothelial cells [51, 58, 99]. The identity of the gene and peptide sequence of this substance, which was named *endothelin* due to its origin, was ultimately revealed by Masaki's group from Japan and was published in 1988 [8, 142]. Subsequently, other members of this peptide family such as endothelin-2 and endothelin-3 were identified [7]. Through the activation of ET_A receptors, endothelin-1 causes sustained and powerful vasoconstriction and also stimulates cell proliferation [7] and mediates endothelium-dependent contractions via *thromboxane* A_2 [122]. Most recently, it was shown that endothelial cell-derived ET-1 is responsible for the majority of the endothelin tissue expression, as endothelial cell-specific preproendothelin-1-deficient mice exhibit a reduction of ET-1 tissue levels in several organs up to 70% compared with wild-type mice [70]. The hypotension observed in these animals also indicates that the vasoconstrictor activity outweighs the dilator activities of endothelin. Like endothelin, angiotensin II is also produced by endothelial cells and through the activation of AT_1 receptors has similar vasoconstrictor and growth-promoting effects if its production increases abnormally [41].

Oxidative stress and inflammation: brothers in arms

Generally, either increasing cellular antioxidant capacity or reducing oxidative stress will have similar beneficial effects on the vasculature (Fig. 1). Due to the fact that NO is

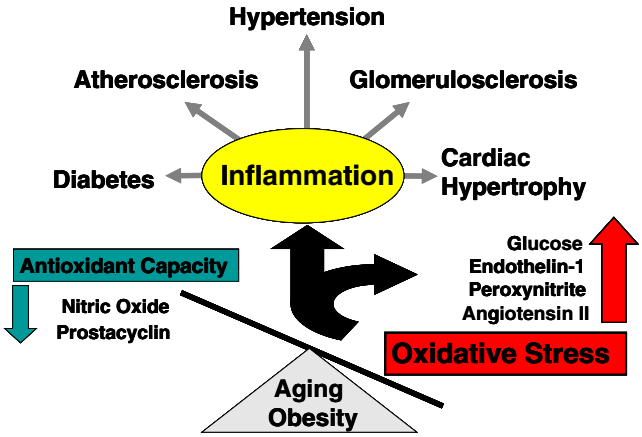

Fig. 1 Comparable and potentiating effects of the risk factors obesity and aging on antioxidant capacity (*left*) and oxidative stress (*right*). Both, obesity and aging, lead to a reduction in formation of bioactive NO and prostacyclin. This is further aggravated by increased levels of glucose, endothelin-1, peroxynitrite (formed from the diffusion-limited reaction between superoxide anion and NO), and angiotensin II. As indicated in the figure, this imbalance favors inflammation as a "common denominator" and thus the development of cardiovascular, renal, and metabolic diseases

formed by the multi-enzyme complex NO synthase [45], which concomitantly produces reactive oxygen species through its NADPH oxidase domain, increasing NO bioactivity has been complicated by NO synthase uncoupling [73]. Since the reaction between NO and superoxide anion is essentially diffusion limited, substantial amounts of peroxynitrite ($ONOO^-$) are formed [5] (Fig. 1). $ONOO^-$ causes cell injury through nitrosylation of proteins, which partially or completely inactivates them [1]. Nitrosylation of proteins, which will cause relatively stable nitrotyrosine to be formed, will change the function, structure, and thus, the accessibility of these proteins to interact with other proteins [94]. Beneficial effects of interventions to reduce oxidative stress and inflammation have been shown, among others, for diseases such as atherosclerosis, myocardial infarction, stroke, peripheral vascular disease, arterial hypertension, chronic renal failure, pulmonary arterial hypertension [134], and for a number of disease conditions mainly associated with chronic inflammation such as connective tissue diseases and metabolic conditions such as insulin resistance and diabetes (Fig. 1).

Current and future world demographics of aging and obesity

Over the past centuries, scientists have developed hundreds of theories to explain the aging phenomenon, many of which are based on the notion that age-dependent changes accumulate with time [4]. Due to last century's economic and scientific advances, aging is not the most frequent

cause of death after age 28 [5] as we have managed to live much longer than our ancestors with an average age of currently around 80 years [5]. The physiological aging process is associated with changes in cellular function, metabolic rearrangements, and structural changes in many organs such as the vasculature, the kidney, the brain, and the heart just to name a few. Ten years from now, the majority of deaths worldwide will have a cardiovascular cause, and within the next 40 years, substantial increases in the aged populations are to be expected [5]. Moreover, by 2050 the world's population are expected to increase by 50% to approximately nine billion [5]. This increase, which will be predominately due to the increased longevity [5], will result in aging of the overall world population [5, 52], and include more than one billion postmenopausal women [12] with a high percentage of obese individuals [5, 12]. It will, therefore, be important to control diseases that occur at an increased incidence with aging. Aging not only promotes the development of atherosclerotic vascular disease, but is also associated with significant metabolic changes, resulting in age-dependent increases of body weight, changes of insulin sensitivity, as well as changes in lipid metabolism [4, 5]. Moreover, the prevalence and incidence of hypertension increases in the elderly [12], which is in part due to arterial stiffening [72, 85, 86] and the arterial calcification associated with it [61]. In this regard, arginase—possibly through interactions with BH_4—has been recently proposed as a new therapeutic target to counteract arterial stiffening associated with aging [69, 113]. Since the above changes directly contribute to atherosclerotic burden, it would be possible to explain the increase in vascular disease seen with aging at least in part by these disturbances. That aging is indeed an independent risk factor for coronary artery disease and stroke is perhaps best evident from patients suffering from Werner syndrome or Hutchinson–Gilford progeria. These patients experience much accelerated aging [84, 89, 135] and usually die within 20 years due to myocardial infarction or stroke [68, 108]. A causal link between defective lamin genes and accelerated vascular aging in these conditions has been recently demonstrated [91, 103].

Aging-associated vascular changes: role of endothelial factors

Aging affects many pathways involved in cardiovascular homeostasis and particularly the function of endothelial cells. In fact, endothelial aging is associated with abnormalities in endothelial cell size and shape [57], susceptibility to apoptosis [62], angiogenesis [106], changes in ploidy and telemore length [2], and abnormal release of vasoactive factors [42, 78]. Overall, the balanced released of factors is tipped towards inflammatory activation and cell growth (Fig. 1). In addition, a number of physiological cardiovascular functions change with increasing age [23, 45].

Mechanisms of endothelial cell dysfunction in aging

Nitric oxide

With age, a number of changes occur in the cardiovascular system that can be considered pro-atherogenic. For instance, the bioactivity of NO decreases and has been considered to be one of the factors contributing to the higher incidence of arterial hypertension, atherosclerosis, and renal disease in aged individuals [4, 83]. Age-related reductions in endothelium-dependent dilation and in NO bioactivity have been shown by Zeiher and coworkers as well as Taddei et al. [123, 144]. We have previously measured, using porphyrinic microsensors, the release of NO from the aortic endothelium of aged rats. We observed that with advanced age in rats—that do not develop atherosclerosis—the stimulated release of NO was reduced by almost 70% [131] (Fig. 2a). This was associated with an attenuation of endothelial dependent relaxation in the same arterial vessel [13, 131]. In contrast to the aorta of rats, endothelium-dependent relaxation to acetylcholine remains unaffected by aging in the femoral artery [13]. We not only studied stimulated NO release [13, 131], but we also found that basal NO release was reduced [13]. Interestingly, the expression of endothelial NO synthase (NOS3) increases with age [54], as does the constitutively expressed inflammatory NO synthase (NOS2) [54] (Fig. 2b). Aortic protein expression of NOS2 and NOS3 also increases with age [31]. The sources of increased O_2^- production in aging arteries are not only enzymes such as NADPH oxidase [65], but also uncoupled NO synthase, which lead to increased formation of O_2^- through its NADPH oxidase domain and possibly involves changes in BH_4 availability [143]. An interesting study in the longest living rodent, which has a life expectancy of up to 26 years compared with 3 to 4 years in other rodents, found that the vasculature of this particular animal expresses much higher amounts of antioxidant enzymes such as SOD [36], suggesting that cellular (but not dietary) antioxidant capacity may indeed be a line of defense against aging-associated cumulative oxidative injury [23, 24]. Finally, other mechanisms such as EDHF appear to take over functions normally attributed to NO with aging in certain vascular beds [50]. Interestingly, in Klotho mice, which show an aging phenotype, only the bioactivity of NO but not prostacyclin is reduced [96].

Endothelin

We found that with aging preproendothelin-1 mRNA expression increases in the vasculature (Fig. 2b) and also

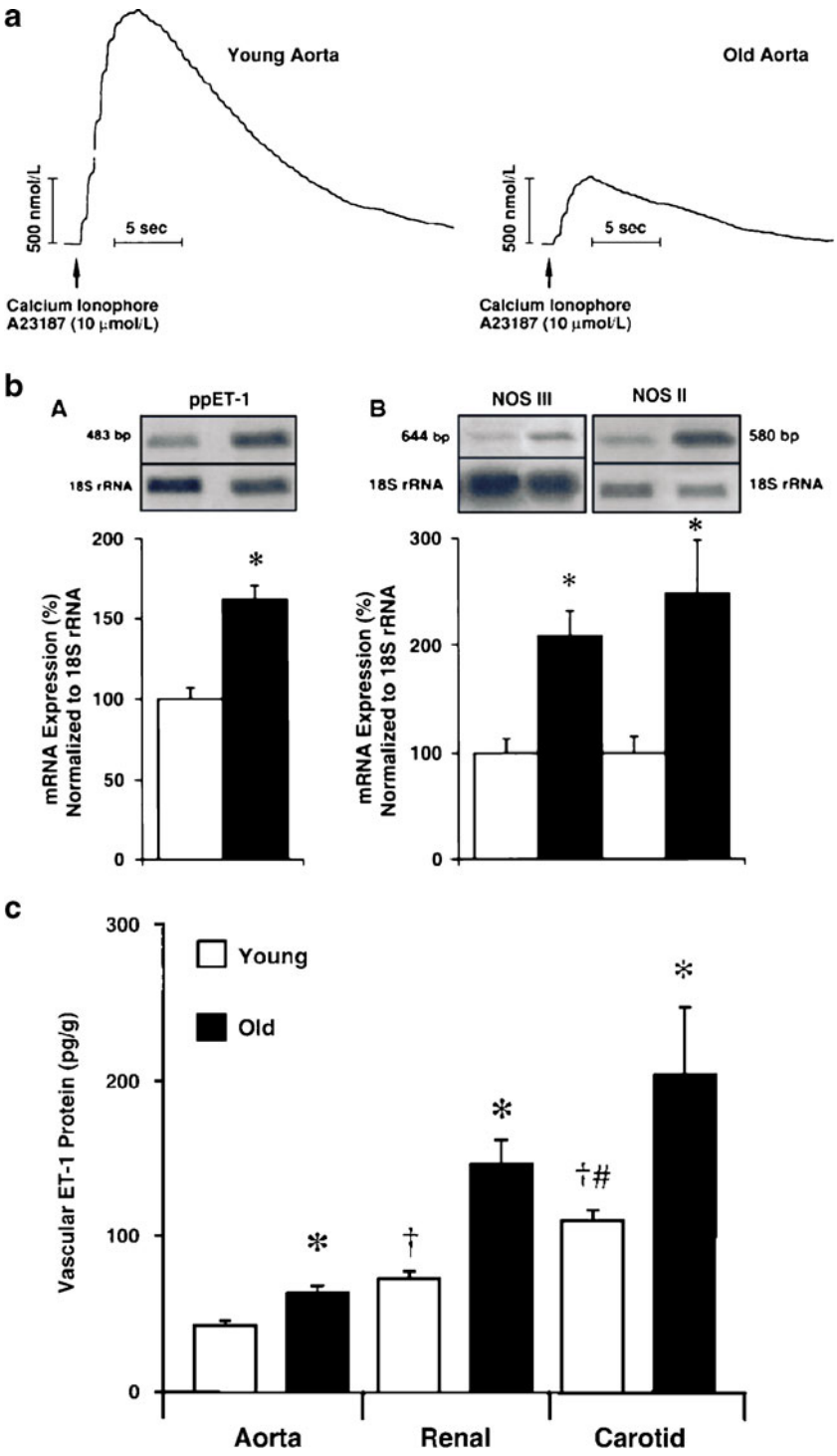

Fig. 2 **a** Effects of aging on vascular NO bioactivity as measured by amperometry in aortic endothelium of rats aged 6 and 33 months of age. Aging was associated with a dramatic decrease in endothelial NO bioactivity. **b** Effects of aging on the vascular expression of preproendothelin-1 gene and genes of NOS3 (endothelial cell NOS) and NOS2 (inflammatory NOS). In the endothelium-intact vascular preparations of rat aorta an up-regulation of all three genes investigated was observed, *$p<0.05$ vs. *young*. **c** Anatomic heterogeneity of endothelin-1 peptide expression between the aorta, renal artery, and the carotid artery in young rats (3 months of age, "young"). At 24 months of age, "old" endothelin-peptide levels as measured by RIA and HPLC increased in all three vascular beds investigated, †$p<0.05$ vs. *aorta*, $p<0.05$ vs. *renal* artery, *$p<0.05$ vs. *young*. Figure in part reproduced from Tschudi et al. [131] (*Panel* **a**) and Goettsch et al. [54] (*Panels* **b** and **c**), with permission from the American Society of Clinical Investigation and the publishers

in the kidney [54, 75]. Interestingly, endothelin-1 peptide expression markedly differed between the aorta, renal, and carotid artery, in all of which an increase of endothelin-1 peptide expression was found with aging (Fig. 2c) [54, 75]. To determine whether endogenous endothelin plays a role for cellular aging and functional injury (proteinuria) in the kidney *in vivo*, we investigated the effects of the blockade of endothelin ET_A receptors in the model of established focal segmental glomerulosclerosis [101]. Although it was previously thought that glomerulosclerosis due to aging is an irreversible process, we unexpectedly found that endothelin inhibition not only reversed proteinuria but that it actually induced partial healing of the previously sclerotic glomerulus [101].

These changes were completely independent of blood pressure and renal hemodynamics and indicated that, indeed, endothelin plays a causal role for the structural and functional changes in the aging cardiovascular system, most likely through its trophic effects [6]. Recent evidence further supports a role for endothelin participating in aging-associated vascular functional injury [39] and enhanced vasoconstriction seen with aging [17, 53].

Obesity as a cause of abnormal production and function of endothelial factors

Within a decade, obesity has become one of the most relevant health issues in many countries around the world [9, 88], with the associated health costs exploding [32]. In 2005, 1.6 billion adults worldwide were overweight and 400 million were obese. By 2015, the numbers are expected to increase even further to 2.3 billion adults being overweight and 700 million obese [79, 98, 115, 119, 120]. In both cases, these numbers do not include children and adolescents, in which obesity also has become a worldwide problem [77]. The reasons for this development are economic growth in developing countries as well and as changes in nutritional patterns combined with the availability of inexpensive and unbalanced diets rich in carbohydrates and fat [25, 79, 88, 98, 115, 119, 120]. Frequently, this is combined with unfavorable lifestyles that particularly include lack of physical exercise and consumption of high-caloric beverages and soft drinks [9, 79, 98, 115, 119, 120]. It has been even proposed that because of the continuing increase of obesity that life expectancy might decline by the middle of this century [100, 119]. One of the most worrisome developments is that obesity now increasingly affects school children [67] who, at a young age, present with diseases normally found only in adults of higher age namely, arterial hypertension and diabetes mellitus [9]. In fact, overweight children already prematurely develop abnormal endothelial cell dysfunction and arterial intima-media thickening [141] normally found in obese adults [118]. This already illustrates that obesity may actually mimic aging in certain aspects. The mechanisms involved in the pathophysiology of obesity are numerous [138]. Mechanisms include, abnormal changes in insulin sensitivity, dyslipidemia, increased vasomotor tone, structural abnormalities in the liver (non-alcoholic steatohepatitis), increased sympathetic drive, structural changes in the kidney, and perhaps most importantly, inflammation [138]. Excessive visceral fat is one of the major contributors of these abnormalities, and studies in rodents and in monkeys indicate that either removal of visceral fat or caloric restriction can extend the lifespan in mammals [34, 95].

Mechanisms of endothelial cell dysfunction in obesity

Nitric oxide

Several studies in experimental animals and humans have shown that in obesity the bioactivity of NO is reduced [18, 22, 37, 104]. The mechanistic concept that has been mostly propagated is the inactivation of NO by superoxide anion (O_2^-), leading to the formation of peroxynitrite (Fig. 1). The source of increased O_2^- production is not only enzymes such as NADPH oxidase, but also uncoupled NO synthase [44, 82]. Increased nitrotyrosine formation as a consequence of peroxynitrite production has been described in obese animal models [22, 26, 48]. More recently, other pathways such as guanylate cyclase, the intracellular target of NO (Table 1), have also been shown to be affected by obesity and have been directly linked to inflammation [107].

Endothelin

Experimental studies suggest that animal models exhibit many of the changes seen with obesity in humans, including inflammation, dyslipidemia, and abnormal vasomotor tone [33, 121, 130].

One of the most important factors responsible for the high prevalence of obesity is an increased intake of high-calorie food rich in carbohydrates and fat [37]. There are a number of excellent experimental models of diet-induced obesity in which changes in the vasculature and kidney have been studied [33, 121, 130]. One of our first efforts in the field was to study the effects of high-calorie, high-fat-diet-induced obesity on the renin–angiotensin system and the mouse kidney [14]. We found that obesity increases activity of the angiotensin converting enzyme (ACE) in the kidney and that this regulation is dependent on endothelin ET_A receptors (Fig. 3). These data suggested that—under certain conditions such as obesity—endothelin receptor antagonists also have ACE-inhibitor functions. We also demonstrated that vascular contractility to endothelin increases both in models of diet-induced obesity and in monogenetic leptin deficient obesity with differences between vascular beds [20, 92, 93, 127, 128]. In addition to being a vasoconstrictor, endothelin-1 is a potent pro-atherogenic peptide [11, 16]. As seen in aging arteries—vascular expression of endothelin at the mRNA level and that of ET_A receptors increases in diet-induced obesity [93, 128] (Fig. 4c, d). Experimental studies provide evidence that diet-induced obesity exerts specific changes promoting enhanced vasoconstriction and arterial hypertension as can be seen in obese humans with regard to an activated endothelin pathway [29]. Clinical studies support this notion and suggest possible

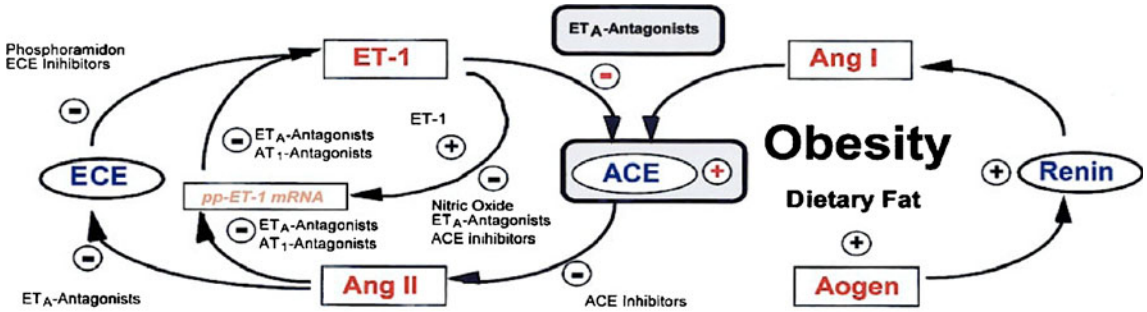

Fig. 3 Effects of obesity on the interactions between tissue RAS and the ET system. Obesity activates components of the RAS in adipose tissue, thereby increasing formation of Ang II. Obesity also increases expression/activity of prepro-ET-1 (ppET-1). Endothelin-converting enzyme (ECE) and endothelin-1 is stimulated by Ang II in vivo, thereby increasing production of ET-1. As shown (shaded boxes), ET-1 also stimulates tissue ACE activity, via activation of ET_A receptors. Expression of ppET-1 mRNA is further regulated by ET-1 in an autocrine manner and by NO. Aogen indicates angiotensinogen, (−) inhibition, (+) stimulation. Figure reproduced from Barton et al. [8] with permission from the American Heart Association and the publisher

therapeutic potential for endothelin receptor antagonists in patients with obesity and related complications such as arterial hypertension [16]. In fact, two clinical studies in obese patients (suffering either from arterial hypertension or diabetic nephropathy) have been most recently published, both showing the beneficial effects of endothelin receptor blockade on renal function and blood pressure [80, 139].

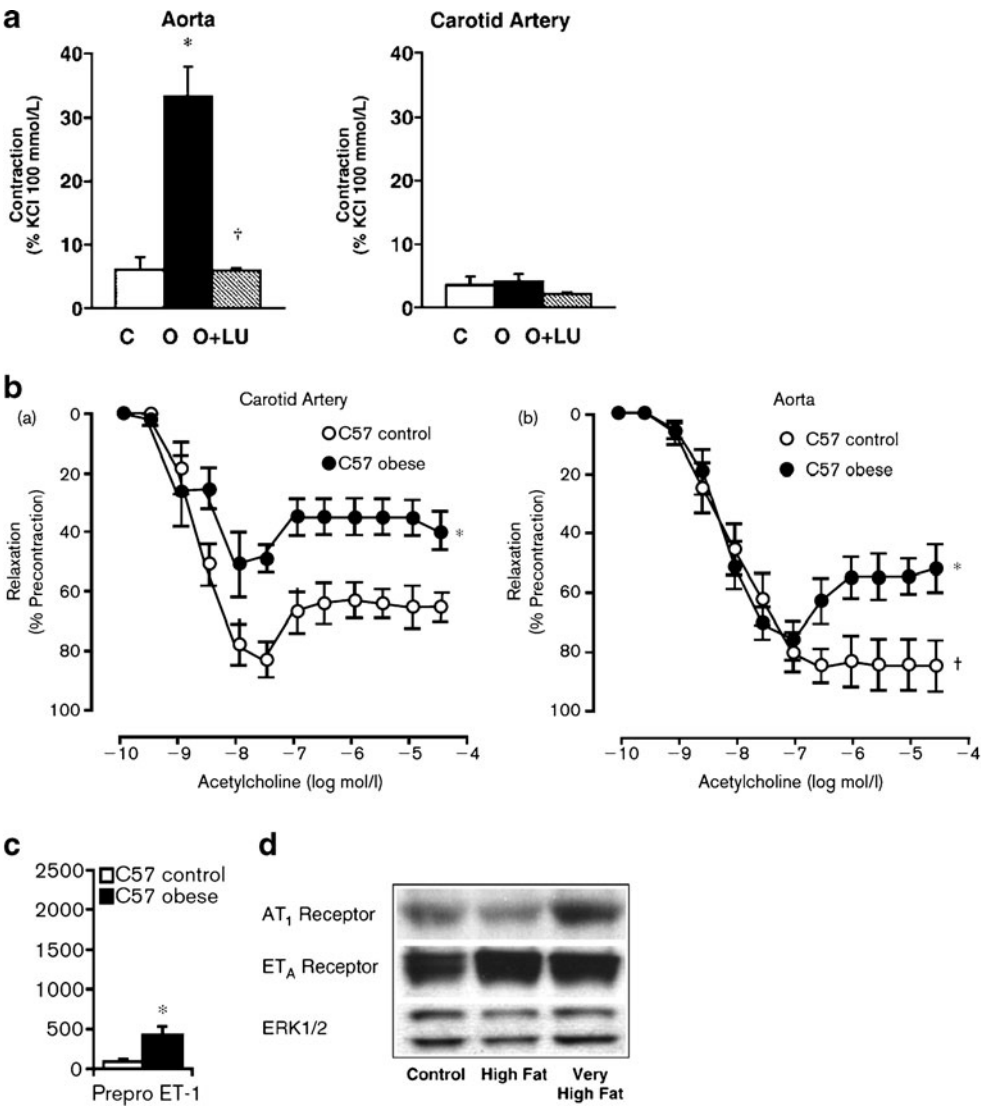

Fig. 4 **a** Promoting effect of diet-induced obesity (o) on angiotensin II-induced contractions in the aorta (*left panel*), but not the carotid artery (*right panel*) in mice. Obesity-induced increases in contractility were completely prevented by in vivo treatment with an orally active endothelin antagonist (darusentan, LU135252, LU) despite continued obesity (O+LU). *$p<0.05$ vs. lean control (*C*), †$p<0.05$ vs. obese (o). **b** Diet-induced obesity in mice (*filled circles*) enhances endothelium-dependent contractions in carotid artery (*left panel*) and aorta (*right panel*). *$p<0.05$ vs. C57 control **c** Up-regulation of diet-induced obesity of the preproendothelin gene in mice following diet-induced obesity. **d** Effect of increasing dietary fat content on vascular expression of the angiotensin AT_1 receptor and the endothelin ET_A receptor in a mouse model of diet-induced obesity. Figure panels in part reproduced from Barton et al. [8] (*Panel* **a**), Traupe et al. [129] (*Panels* **b** and **c**), and Mundy et al. [93] (*Panel* **d**). Reproduced with permission from the American Heart Association and the publishers

Angiotensin

Similar to what can be seen during aging [17] obesity does not equally affect all vascular beds to the same degree. Using the C57 mouse model of diet-induced obesity [121], we found that contractions to angiotensin II markedly increased only in the aorta, but not in the carotid artery [14] (Fig. 4a). Most surprisingly, we found that the increased contractility was completely prevented if animals were concomitantly treated with an endothelin ET_A receptor antagonist [14]. These effects were independent of body weight and arterial blood-pressure, suggesting that endogenous endothelin is activated during obesity and that it contributes to angiotensin-mediated vasoconstriction in selected vascular beds. Contractions to angiotensin and this model were also blocked by cyclooxygenase inhibition in vitro to a large degree, suggesting that in the mouse vasculature endothelial EDCFs formed from vasoconstrictor prostanoids contribute to the contractility of other vasoconstrictors [14], an effect that may be age-dependent [71]. Interestingly, the amount of dietary fat content affects aortic protein expression of the AT_1 receptor, which was found to be up-regulated only if the diet contained very high amounts of fat [93] (Fig. 4d).

Vasoconstrictor prostanoids

Enhanced vasoconstriction has been observed in patients with obesity [117] and both cyclooxygenase and endothelin has been implicated in these responses. In obese mice endothelial vasoconstrictor prostanoids are increasingly formed in both aorta and carotid artery (Fig. 4b) [128]. Contractions are sensitive to blockade with nonselective COX inhibition, but not COX-2 selective inhibitors [128]. In a simple and elegant study it was subsequently shown by Vanhoutte's group using COX-1 and COX-2 deficient mice that COX-1 is indeed the enzyme responsible for prostanoid-mediated EDCF production in mice [124]. Our results suggest that with obesity, COX-1 dependent vasoconstrictor pathways become activated and that they contribute to enhanced vasoconstriction as can be seen in obese humans [104]. Again, a similar activation of COX-dependent pathways has been reported to occur with aging [125], which is yet another similarity between the two conditions. Indeed, our recent work comparing functional vascular injury due to obesity in youth and adulthood suggests that obesity indeed causes changes compatible with accelerated, "premature" functional vascular aging [19]. Aside from COX-derived EDCFs, another endothelium-derived arachidonic acid product, prostacyclin, has recently been directly implicated in obesity, by determining the fate for the development of fat cells from progenitor cells [64, 136].

Hydroxyl radical

The role of hydroxyl radical in vascular biology has not been investigated much. We have analyzed the production of hydroxyl radical in normal mice and in monogenetic obesity [92]. We found that endothelin-1 stimulates hydroxyl radical formation and that obesity, more or less, abolishes the stimulating effect of endothelin on hydroxyl radical formation [92]. On the other hand, the relaxant response to hydroxyl radical was enhanced in animals with monogenetic obesity [92]. Similar observations were made in models of diet-induced obesity, where vascular responses to hydroxyl radical changed from contractions in lean animals into relaxations upon treatment with high-fat diet, again effects being specific to a certain vascular beds [20].

"Endothelial therapy" for and aging obesity

Atherosclerosis is a systemic, age-dependent inflammatory vascular process that still accounts for half of the morbidity and mortality in industrialized countries [15]. Atherosclerosis is associated with age-dependent coronary vascular calcification, which shows a gender difference affecting women much less than men [61, 114]. In atherogenesis, inflammation—most likely due to and further augmenting oxidative stress—is one of the main pathophysiological mechanisms propagating disease progression [10] and has been directly implicated in vascular calcification [116]. Early lesions of the atherosclerotic plaque (fatty streaks) consisting of endothelial deposits of lipid-laden macrophages, can be detected in the fetal aorta, and their progression is aggravated by maternal hypercholesterolemia [97] and age. This suggests that lipids are required for disease onset and progression of atherosclerosis already early in life. Importantly, already in children, obesity promotes the development of fatty streaks and coronary atheromata, pathological changes from which surprisingly, girls appear to be protected due to endogenous estrogen production [87].

Despite the lack of scientific evidence, the pharmaceutical and cosmetics industry continues to devote much activity to the economically rewarding field of aging "prevention" (rejuvenation), often also called "anti-aging". There are now even scientific journals dedicating their efforts exclusively towards "rejuvenation" [35]. Despite a general desire for "rejuvenations" that is largely fueled by psychological and social factors, efforts should not be focused on finding potions and remedies [4, 5]. Instead of trying to "turn back time", aging can and should be accepted as a physiological process that does not require intervention but allows a life very worthwhile if the right steps are taken in due time [4, 5]. Not surprisingly, in

elderly humans endothelium-dependent vasoreactivity can be preserved by exercise even at an older age [66]. However, aged individuals frequently exhibit conditions favoring the development of hypertension, dyslipidemia, and atherosclerosis, including a high prevalence of obesity, lack of exercise, and unfavorable dietary regimens [9]. Unfortunately, these conditions are no longer limited to aged individuals, but already present to a considerable degree in children [9, 77]. It will thus require timely and powerful interventions if we want to avoid future disease in adulthood and even later in life. In fact, childhood obesity—even if normal body weight is maintained in later life—increases the likelihood of adult coronary artery disease [3, 21, 77].

A decade ago we proposed the concept of "endothelial therapy" as a means to preserve and/or improve function and reduce production of deleterious endothelium-derived mediators to interfere with atherosclerosis progression [8]. A number of modalities are available to interfere with age-related changes in endothelial cell function [66]. Preventive measures, which apply to children and adolescents as well, include cessation of smoking, normalization of increased body weight, and avoiding unbalanced diets rich in fat and sugars and low in fibers [30]. Interestingly, nutritional additives such as vitamins appear to be largely ineffective to interfere with age-dependent functional changes [4, 5]. As aging is frequently associated with a reduction of physical activity and fitness, it is even more important to emphasize the "therapeutic" role of regular physical activity, which also helps reduce the incidence and improve the severity of related co-morbidities such as diabetes, high blood pressure, dyslipidemia, and obesity [4, 5]. In fact, it has been demonstrated that lack of exercise accelerates most diseases known to show an increased prevalence with aging [76]. Most recent work from Lauf's group suggests that exercise can actually slow down vascular aging [140]. It can be anticipated that maintaining or even improving cardiovascular health with age is not only likely to result in improved general health, but can also be expected to have a positive impact on cardiovascular and renal morbidity and mortality [15, 129], and that it would result in enormous economic benefits for health systems worldwide. Indeed, regular intense exercise has beneficial effects on cardiovascular health showing a dramatic risk reduction [81] that appears to be equally effective in obese individuals. Similarly, weight loss has been shown to improve the vascular risk profile by reducing aortic pulse wave velocity [105].

Changes aiming to achieve normal body weight and improved fitness of the world population will require timely implementation and it also will provide us with a chance to further study endothelial cell biology in the clinical setting more closely in the context of obesity and aging. However, we must not wait too long to make these changes work. Should we fail to reach the required goals it is well possible that we might—for the first time—experience a decline in the longevity that we have achieved over hundreds of years [100, 119].

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